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(54) METAL THIN FILM FOR SPR SENSOR, ITS MANUFACTURE AND MEASURING METHOD USINT IT

(57)Abstract:
PROBLEM TO BE SOLVED: To provide a sensor device by which more living-body components can be detected by a method wherein the surface of a sensor is chemically modified by using a polymer which has various characteristics and functions.
SOLUTION: This metal thin film is formed in such a way that (1) the surface of a metal thin film which has a free-electron metal surface is treated with an organic linker which has a functional group capable of being immobilized to a metal surface and a functional group capable of being bonded to a polymer and that (2) it is treated with a polymer which has a functional group capable of being bonded directly to a reagent for detection or a functional group capable of being bonded to the reagent for detection via a spacer and a functional group capable of being bonded to the organic linker. In more detail, the polymer refers to a polymer which has an amino group such as chitosan, polyethleneimine or the like.

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CLAIMS

[Claim(s)]

[Claim 1] the polymer which has the functional group which can combine with the functional group which the front face of the metal thin film which has a free electron surface of metal can combine with the functional group or the reagent for detection which can couple directly with the reagent for (2) detection through a spacer at the organic linker which has the functional group which can be combined with the functional group and the polymer which may be fixed to (1) surface of metal, and a list, and said organic linker, and the metal thin film which it comes and comes be processed out of.

[Claim 2] The metal thin film according to claim 1 which is the shape of a straight chain, the shape of branching, and the annular chemical structure whose straight chain part between the functional groups which can be combined with the functional group which may be fixed to the surface of metal of an organic linker, and a polymer consists of a carbon atom, an oxygen atom, and an atom chosen from the group which consists of a nitrogen atom, and is two to 20 atom.

[Claim 3] The metal thin film according to claim 1 or 2 which two or more sorts of organic compounds combine [an organic linker].

[Claim 4] The metal thin film according to claim 1 to 3 whose functional group which may be fixed to the surface of metal of an organic linker is a functional group containing a sulfur atom.

[Claim 5] The metal thin film according to claim 4 whose functional group which may be fixed to the surface of metal of an organic linker is a sulfhydryl group, a sulfide radical, or a disulfide radical.

[Claim 6] The metal thin film according to claim 1 to 5 which is the amino acid with which the organic compound which has the functional group which forms an organic linker and may be fixed to a surface of metal contains the functional group containing a sulfur atom, or its derivative.

[Claim 7] The metal thin film according to claim 6 whose organic compound which has the functional group which forms an organic linker and may be fixed to a surface of metal is a cysteine or its ester derivative.

[Claim 8] The metal thin film according to claim 1 to 7 whose functional groups which can be combined with the polymer of an organic linker are a carbonyl group, the amino group, carboxyl groups, or these derivatives.

[Claim 9] The metal thin film according to claim 1 to 8 whose organic linkers are a cysteine or its ester derivative, and the thing guided from glutaraldehyde.

[Claim 10] The metal thin film according to claim 1 to 9 whose organic linker is the fatty acid of the carbon numbers 2-20 which have a sulfhydryl group, a sulfide radical, or a disulfide radical.

[Claim 11] The metal thin film according to claim 1 to 10 whose functional groups which can be combined with the functional group or the reagent for detection which can be coupled directly with the reagent for detection of a polymer through a spacer are the amino group, a carboxyl group, hydroxyl groups, or these derivatives.

[Claim 12] The metal thin film according to claim 11 whose polymer is a polymer which has an amino group in the principal chain or side chain of a polymer.

[Claim 13] The metal thin film according to claim 12 whose polymer is polysaccharide containing aminosugar.

[Claim 14] The metal thin film according to claim 13 whose polymer is chitosan.

[Claim 15] The metal thin film according to claim 12 whose polymer is a Pori low-grade alkylene imine.

[Claim 16] The metal thin film according to claim 1 to 15 by which only one side of a metal thin film is processed.

[Claim 17] The metal thin film according to claim 1 to 16 whose thickness of the thin film of a metal thin film is 50-1000nm.

[Claim 18] The metal thin film according to claim 1 to 17 whose metal of a metal thin film is any one sort of gold,

silver, copper, aluminum, or chromium.
[Claim 19] The metal thin film according to claim 18 whose metal of a metal thin film is gold.
[Claim 20] The metal thin film according to claim 1 to 19 which the reagent for detection has combined with the polymer through the spacer which has further the functional group which the reagent for detection can combine with the reagent for direct or detection.
[Claim 21] The metal thin film according to claim 20 which is the organic compound which has the functional group which a spacer can combine with the functional group and the reagent for detection which can be combined with a polymer.
[Claim 22] The metal thin film according to claim 21 whose functional group which can be combined with the reagent for detection is a carboxyl group or its reactant derivative.
[Claim 23] The metal thin film according to claim 20 to 22 whose reagent for detection is protein.
[Claim 24] The metal thin film according to claim 23 whose protein is an antigen or an antibody.
[Claim 25] The metal thin film according to claim 1 to 24 which the metal thin film has stuck to the substrate.
[Claim 26] The metal thin film according to claim 25 whose substrate is prism.
[Claim 27] The metal thin film according to claim 1 to 26 whose metal thin film is a metal thin film for surface plasmon resonance (SPR).
[Claim 28] It processes with the first organic compound which has the functional group and other functional groups which may be fixed to a surface of metal in the front face of the metal thin film which has a free electron surface of metal. The second organic compound which can carry out the functional group and chemical reaction of the others which fix the first organic compound concerned to a surface of metal, and are not being fixed subsequently to the surface of metal of the first organic compound concerned is made to react. How to manufacture the metal thin film with which the organic linker which consists of combining said the first organic compound and second organic compound concerned was introduced.
[Claim 29] The approach according to claim 28 of being the amino acid with which the first organic compound contains the functional group containing a sulfur atom, or its derivative.
[Claim 30] The approach according to claim 29 the first organic compound is a cysteine or its ester.
[Claim 31] The approach according to claim 28 to 30 the second organic compound is glutaraldehyde.
[Claim 32] The organic linker which has the functional group which can be combined with the functional group and polymer which may be fixed to (1) surface of metal is introduced into the front face of the metal thin film which has a free electron surface of metal. Subsequently (2) Consist of making the polymer which has the functional group which can be combined with the functional group which can be combined with the functional group or the reagent for detection which can be coupled directly with the reagent for detection through a spacer, and said organic linker react with said organic linker, and combining this. How to manufacture the metal thin film by which the front face was processed.
[Claim 33] The approach according to claim 32 a polymer is a polymer which has an amino group in the principal chain or side chain of a polymer.
[Claim 34] The approach according to claim 33 a polymer is the polysaccharide or the Pori low-grade alkylene imine containing aminosugar.
[Claim 35] The approach according to claim 34 a polymer is chitosan or polyethyleneimine.
[Claim 36] The organic linker which has the functional group which can be combined with the functional group and polymer which may be fixed to (1) surface of metal is introduced into the front face of the metal thin film which has a free electron surface of metal. The polymer which has the functional group which can be combined with the functional group which can be combined with this through a spacer at the functional group or the reagent for detection which can be coupled directly with the reagent for (2) detection, and said organic linker is made to react with said organic linker, and this is combined. Subsequently, further How to manufacture the metal thin film by which the front face to which mind [concerned] the spacer which has the functional group which can be combined with the reagent for direct or detection, and make the reagent for detection join together or come to stick was processed.
[Claim 37] The approach according to claim 36 of being the organic compound which has the functional group which a spacer can combine with the functional group and the reagent for detection which can be combined with a polymer.
[Claim 38] The approach according to claim 36 or 37 the functional group which can be combined with the reagent for detection is a carboxyl group or its reactant derivative.
[Claim 39] The approach according to claim 36 to 38 the reagent for detection is protein.

[Claim 40] The approach according to claim 39 protein is an antigen or an antibody.
[Claim 41] The approach according to claim 28 to 40 a metal thin film is gold, silver, copper, aluminum, or chromium.
[Claim 42] They are detection, identification, or the approach of carrying out a quantum about a sample by the surface plasmon resonance (SPR) characterized by using the metal thin film with which the metal thin film for surface plasmon resonance (SPR) measurement is beforehand processed by surface plasmon resonance (SPR) by the approach according to claim 28 to 41 in the sample detection, identification, or in case a quantum is carried out.
[Claim 43] They are detection, identification, or the approach of carrying out a quantum about a sample by the surface plasmon resonance (SPR) characterized by processing the metal thin film for surface plasmon resonance (SPR) measurement for a sample during measurement by the approach according to claim 28 to 41 by surface plasmon resonance (SPR) detection, identification, or in case a quantum is carried out, and measuring by subsequently adding a sample.
[Claim 44] They are detection, identification, or the approach of carrying out a quantum about a sample by the surface plasmon resonance (SPR) characterized by using a metal thin film according to claim 1 to 27.
[Claim 45] To the metal thin film which combined the spacer which has the functional group which can combine the reagent for detection with a metal thin film according to claim 1 to 19 or its polymer part, the reagent for detection and a sample are added at sequential or coincidence, and they are detection, identification, or the approach of carrying out a quantum by surface plasmon resonance (SPR) about the trend of the reagent for detection, and the matter in a sample.
[Claim 46] The approach according to claim 45 the trend of the reagent for detection and the matter in a sample is the antigen antibody reaction.
[Claim 47] (1) They are detection, identification, or a kit for carrying out a quantum about a sample by the polymer which has the functional group which can be combined with the functional group which can be combined with the organic linker which has the functional group which can be combined with the functional group and the polymer which may be fixed to a surface of metal, the functional group which can be coupled directly with the reagent for (2) detection, or the reagent for detection through a spacer, and said organic linker, and the surface-plasmon resonance (SPR) which consists of a (3) metal thin film.
[Claim 48] Furthermore, the kit according to claim 47 which comes to include the spacer for combining this with a polymer from that of the reagent for (4) detection, and the need.
[Claim 49] Surface plasmon resonance (SPR) equipment which used the metal thin film according to claim 1 to 27.

[Translation done.]

DETAILED DESCRIPTION

[Detailed Description of the Invention]
[0001]
[Field of the Invention] This invention relates the metal thin film by which surface treatment was carried out by the organic linker which the polymer combined, the surface plasmon resonance (SPR) equipment using it, and the sample by it to detection, identification, or the approach of carrying out a quantum.
[0002]
[Description of the Prior Art] In the field of inspection of an infectious disease, or a diagnosis, immunoassay is used widely today. Generally, from the usual infectious disease, a lot of antibodies are made by the inside of the body, and it is diagnosing by recognizing the existence of this antibody. Moreover, the value of the components in body fluid, such as a blood serum, urine, and saliva, (blood sugar level, cholesterol, etc.) also serves as an important parameter, when grasping a patient's symptoms and examining a treatment policy. In the case of a diagnosis, the output of a sample has constraint, and since extraction of **** is difficult, a high precision (repeatability) and sensibility are required of a measuring method. And since only the minute amount is usually contained in the complicated matrix of a biological material, the purpose component is very important for obtaining the high analysis result of reliability exactly.
[0003] On the other hand, a surface plasmon resonance (Surface Plasmon Resonance:SPR) sensor is a device which detects the surface plasmon resonance phenomenon generated on metal thin film front faces, such as gold and silver. Surface plasmon is a kind of the wave of expansion of the electron produced in a metal-dielectric interface, and the wave number changes with the thickness and the optical properties (the dielectric constant, refractive index) of a sample to several 100nm which touch a metal thin film front face. Since it is impossible to measure this change directly, laser light is put in a SPR sensor from the opposite side of a sample, an evanescent wave is generated, and it has become a general approach to measure change of a surface condition indirectly by measuring change (SPR angle shift) whenever [incident angle / of laser in case this resonates with surface plasmon].
[0004] The example in which the surface plasmon exciting method by optical pumping was established by KURECHUMAN (Kretschmann) in 1971, and this sensor became 11 years after, and SPR was applied to gas sensing by NAIRANDA and others (Nylander) as a sensor for the first time was reported. Although this sensor was the simple thing which caught refractive-index change of the bulk by existence of a sample, development of this kind for the alcoholic concentration in a sample solution etc. of sensor was briskly performed by the 1990 time.
[0005] Research of the self-adsorption to golden front faces, such as a thiol and sulfides, was done, and the example of research of the self-adsorbent monolayer (Self-Assembled Monolayer) which embellished the golden front face with alkyl thiols with various functional groups, such as a carboxylic acid and an amino group, was reported at the second half of the 1980s. Made another layer which has a charge form in 1993 on the self-adsorbent monolayer on the front face of golden (Self-Assembled Monolayer). The biosensor which fixed to the active site of two-layer structure the ligand which shows specific reactions, such as an antigen-antibody, is developed. It came (Stelzle, M., et al., J.Phys.Chem., 97, and 2974-2981 (1993)) to be used for quality, quantitative analysis, the elucidation of a reaction process, etc.
[0006] Moreover, the example of research of the optical-fiber mold SPR sensor aiming at a miniaturization began to be reported from these days, wavelength of the conventional light source was fixed, and the sensor equipment to which the incident angle of light is uniformly carried out, and wavelength is changed from the approach of measuring the incident angle from which the resonance to a sample takes place was developed. In 1995 and

afterwards, many examples of sensing using a SPR sensor are reported, and many examples of research aiming at high-sensitivity-izing and new ligand measurement of a sensor are reported by especially the field of a biosensor.

[0007] In development of a SPR chemical sensor, in order to require that a certain qualification should be performed to the metal thin film of the sensing section and to make a SPR sensor apply to a biosensor, the qualification to the metal thin film of the polymer which has a functional group can be considered. When using a golden thin film for a metal thin film, the approach of being able to consider the embellishing method for using the thiol to gold and the self-adsorbent (Self-Assembly) of sulfides, actually introducing a carboxymethyl dextran (CMD) layer by making into a linker layer the alkane thiol which carried out self-adsorption on Au thin film at immobilization of the ligand on the front face of a sensor in a SPR biosensor, and fixing in the CMD layer is reported. The roles of a CMD layer are the increment in the amount of ligands to fix, nonspecific joint prevention of the biomolecule to a golden thin film, etc. The qualification approach of a thiol and sulfides is called 90% of surface coverage only by dipping 1h and a golden thin film in the ethanol solution of the thiol of 1mM in ClaireE.Jordan's and others paper (Jordan, C.E., et al., Langmuir, 1994, 10, 3642-3648), and it is possible because evaluation of adsorption looks at [easy] XPS (X-ray photoelectron spectroscopy) and SPR angle change.

[0008] Moreover, invention about a detection front face which comes to prepare the layer of raw compatibility porosity matrices, such as a hydrogel, on the layer combined with a surface of metal is indicated by ****** No. 501605 [four to]. Invention about the sensor unit which uses two functionality or a polyfunctional molecule for ****** No. 501606 [four to] is indicated. Moreover, invention about the decision approach of the property of the macromolecule using the sensor which ligand combined with the sensor front face is indicated by ****** No. 501607 [four to]. Invention about the method of detecting the analysis object which has an interest in a sample using the solid-state base material which contains the fixed joint partner including a reversible joint acceptor in ****** No. 507865 [seven to], and equipment is indicated. Moreover, invention about the manufacture approach on the front face of a disposition which combined film formation protein or a lipid on self-adsorbent monolayer (Self-Assembled Monolayer) is indicated by WO 96/10178.

[0009] Since the part along which the laser light used for measurement passes since it is the device which measures the change of state of a sample indirectly using an evanescent wave differs from the part where a sample exists, a SPR sensor has the following advantages as compared with the conventional photo sensor.

1. Since refractive-index change of the sample which happens on a sensor front face can detect on real time, the advance situation and kinetics-relation of an experiment can be known.
2. Since the laser light used for measurement does not pass through the inside of the sample solution, it is hard to be influenced of coloring of the sample solution, muddiness, air bubbles, etc.
3. Since the optical property in an about 1-micrometer very narrow field is detected from a sensor base front face, there are very few amounts of the sample solution to need, end, and it is hard to be influenced of background noise.

However, the sensibility of SPR was not good, and the reactivity of processing on the conventional front face of a sensor was not enough as it, either, and it was not practical to measurement on real time.

[0010]

[Problem(s) to be Solved by the Invention] This invention is carrying out chemical modification of the sensor front face using the giant molecule which has various properties and functions, and aims at offering the sensor device which can detect more biogenic substances. Using a metal thin film as a sensor base, surface plasmon resonance (Surface Plasmon Resonance:SPR) equipment is used as a device, and it aims concrete at offering the high sensitivity immune sensor using the antigen antibody reaction. Furthermore, it aims at offering the new sugar sensor using sugar joint protein (Concanavalin A:ConA). Moreover, this invention offers detection of the sample using these new metal thin films for sensors, the SPR equipment using it, and it, identification, or the quantum approach.

[0011]

[Means for Solving the Problem] this invention relates to the polymer which has the functional group which can combine with the functional group which the front face of the metal thin film which has a free electron surface of metal can combine with the functional group or the reagent for detection which can couple directly with the reagent for (2) detection through a spacer at the organic linker which has the functional group which can be combined with the functional group and the polymer which may be fixed to (1) surface of metal, and a list, and said organic linker, and the metal thin film which it comes and comes to be processed out of. This invention relates to said metal thin film whose polymer concerned is a polymer which has amino groups, such as chitosan

or polyethyleneimine, more at a detail.

[0012] Moreover, this invention relates to the metal thin film which the reagent for detection has combined with said polymer through the spacer which has further the functional group which the reagent for detection can combine with the reagent for direct or detection. The metal thin film of this invention is useful as a sensor thin film of the high sensitivity for measurement of surface plasmon resonance (SPR).

[0013] Moreover, this invention relates to the manufacture approach of said metal thin film, the measuring method by the surface plasmon resonance (SPR) which used this, the kit for it, and a surface plasmon resonance (SPR) measuring device.

[0014] The front face of the metal thin film of this invention consists of a reagent layer for detection which (1) one side combines with the polymer layer combined with the organic linker layer and (2) organic linker layer which are being fixed to the metal thin film front face, and (3) polymer layers through direct or a spacer.

[0015] Between the functional groups which can be combined with the functional group which may be fixed to a surface of metal, and a polymer the organic linker layer of this invention It consists of a carbon atom, an oxygen atom, and an atom chosen from the group which consists of a nitrogen atom. That a straight chain part should just be what has preferably the shape of a straight chain which is two to 10 atom more preferably, the shape of branching, and the annular chemical structure two to 15 atom two to 20 atom, although you may be single molecular species, it may be designed using two or more sorts of molecular species. Since the functional group which is comparatively rich in reactivity can be used, it is a short time and it comes out to form an organic linker layer cheaply in using two or more sorts of molecular species, it becomes the mode of more desirable this invention.

[0016] Although you may be the functional group fixed by a surface of metal and the chemical bond as "a functional group which may be fixed to a surface of metal" of the organic linker which has the functional group which can be combined with the functional group and polymer which may be fixed to the surface of metal which forms the organic linker layer of this invention, you may be the functional group which may be fixed by adsorption. The thing containing a sulfur atom like a sulfhydryl group, a sulfide radical, or a disulfide radical as such a functional group is desirable. Moreover, various functional groups can be chosen according to the functional group which the polymer to be used contains as "a functional group which can be combined with a polymer." For example, when the polymer contains the amino group, a carboxyl group, a carbonyl group, etc. can be chosen, and when the polymer contains the carboxyl group, the amino group, a hydroxyl group, etc. can be chosen. The carbonyl group in this invention includes an aldehyde group and a keto radical, and is an aldehyde group preferably.

[0017] the carbon numbers 2-20 which have the functional group which contains a sulfur atom like a sulfhydryl group, a sulfide radical, or a disulfide radical in using molecular species single as an organic linker of this invention -- desirable -- 2-15 -- the fatty acid of 2-12 can be used more preferably. 11-mercapto-undecanoic acid is more specifically mentioned.

[0018] What is formed using two or more sorts of molecular species as an organic linker of more desirable this invention is mentioned. The organic compound which has other reactant functional groups which have a functional group containing a sulfur atom like a sulfhydryl group, a sulfide radical, or a disulfide radical as "a functional group which may be fixed to a surface of metal" as the first molecular species fixed to a surface of metal, and can form the second molecular species and chemical bond is mentioned. The amino group, a hydroxyl group, a carboxyl group, etc. are mentioned that what is necessary is just what can form the second molecular species and chemical bond as a reactant functional group. The amino acid which has the functional group which contains a sulfur atom like a sulfhydryl group, a sulfide radical, or a disulfide radical from reactivity or the ease of acquisition, or its derivative is desirable, and cysteine alkyl ester is more specifically desirable. as alkyl ester -- carbon numbers 1-10 -- the alkyl ester of 1-5 is preferably desirable. More specifically, cysteine methyl ester, cysteine ethyl ester, etc. are mentioned.

[0019] It is the organic compound which has the functional group which can carry out a chemical bond to the reactant functional group of the first molecular species as the second molecular species combined with the first molecular species which forms the organic linker of this invention, and has the functional group which can be combined with the polymer which forms the following polymer layer, and the amino group, a carboxyl group, a hydroxyl group, a carbonyl group, etc. are mentioned as these functional groups, for example. The compound which has the carbonyl group which is rich in reactivity as the second desirable molecular species, for example is desirable, and glutaraldehyde etc. is more specifically mentioned. As molecular species which forms the organic linker of this invention, although the combination of various organic compounds exists, as a desirable

combination, using cysteine methyl ester as the first molecular species, the thing using glutaraldehyde as the second molecular species is mentioned, and since a reaction advances without carrying out special activation actuation (for example, a carboxyl group being made into activity ester.), this thing can make a functional group the mode of desirable this invention.

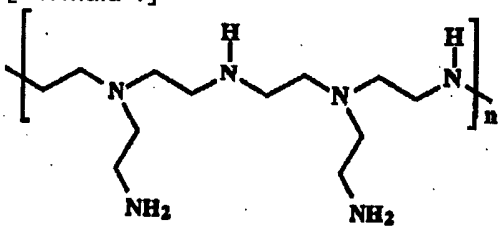
[0020] It can carry out by contacting the compound used as an organic linker to a metal as an approach of fixing the organic linker of this invention to a surface of metal. Before making it contact, it is desirable to wash a surface of metal with alkali, such as a potassium hydroxide. It is desirable to perform contact to a surface of metal to the bottom of existence of organic solvents, such as dimethylformamide (DMF), water, etc. Subsequently, an organic linker layer can be formed by making the second molecular species react if needed. In this case, after activating the functional group which participates in a reaction if needed, it can also carry out.

[0021] It is the polymer which has the functional group which can combine the spacer for having the reactant functional group which can be combined with "the functional group which can be combined with a polymer" in organic linkers, such as an amino group, a carboxyl group, a hydroxyl group, and a carbonyl group, as a polymer which forms the polymer layer of this invention, and combining the reagent for detection, or the reagent for detection. Although "the reactant functional group" in these polymers and "the functional group which can be combined with the reagent for detection etc." may be different functional groups, it may be ******** of the same kind. As a desirable functional group, the amino group, a carboxyl group, a hydroxyl group, a carbonyl group, etc. are mentioned. The polymer to which "the functional group which can be combined with a polymer" in an organic linker has a first-class amino group in the case of carbonyl groups, such as an aldehyde group, is desirable. Although it combines with the principal chain of a polymer, as for the first-class amino group in a polymer, what is combined with the side chain of a polymer is desirable.

[0022] Although a polyalkylene imine, polysaccharide, the poly lysine, etc. will be mentioned if the polymer of this invention is illustrated, the polysaccharide containing a Pori low-grade alkylene imine or aminosugar is desirable. Although a polyalkylene imine has the desirable polymer with which the polymer of alkylene diamine is mentioned and the first-class amino group exists in the side-chain part, the first-class amino group may be introduced into a side-chain part by chemical modification after a polymerization. As alkylene diamine, it is carbon numbers 2-10 and the diamine which has the alkylene group of 2-6 preferably, and ethylenediamine is mentioned preferably. The polyethyleneimine which has the repeat unit shown by the degree type as a polymer of desirable alkylene diamine, for example is mentioned.

[0023]

[Formula 1]



[0024] As polysaccharide, the polysaccharide containing aminosugar is desirable, and that of the polysaccharide aminosugar carried out [polysaccharide] the polymerization like chitosan in which aminosugar carried out the polymerization is also desirable although aminosugar is introduced partially. Reactant functional groups, such as an amino group, may be introduced into **** and polysaccharide. although the molecular weight of these polymers changes also with classes of polymer -- general -- 1,000-1,000,000 -- desirable -- 5,000-1,000,000 -- it is 5,000 to about 500,000 more preferably.

[0025] When reactivity is not enough, you can make it activated by activation, for example, activity esterification etc., and these functional groups can also be made to react as an approach of combining an organic linker and a polymer, although the direct reaction of these can be carried out to the bottom of existence of a solvent. As a source material, the approach of the metal thin film of this invention at the time of using cysteine methyl ester, glutaraldehyde, and polyethyleneimine is shown in drawing 1 . After washing by attaching to the potassium-hydroxide water solution which warmed the metal thin film several times, by dipping in the DMF solution of L-cysteine methyl ester, it considered as the condition of (1) of drawing 1 , and this was able to be processed in the glutaraldehyde water solution, and was able to be changed into the condition of (2) of drawing 1 , and it was able to consider as the sensor probe which dips a polyethyleneimine water solution in this and has the polyethyleneimine film of (3) of drawing 1 </A> .

[0026] Moreover, the approach of the metal thin film of this invention at the time of using cysteine methyl ester, glutaraldehyde, and chitosan is shown in drawing 2 as a raw material. In drawing 2 , alpha-bromoacetic acid is further used as a spacer. By the approach shown in drawing 1 , and the same approach, after washing a metal thin film, it processed in the L-cysteine methyl ester DMF solution and the glutaraldehyde water solution, and the organic linker part was prepared, by contacting a chitosan water solution to this 1%, chitosan was fixed and the sensor probe was prepared. Subsequently, chitosan was carboxylated with the sodium-hydroxide solution of alpha-bromoacetic acid, and CM-chitosan sensor probe which turned carboxymethyl (spacer part) was prepared.

[0027] as a reagent for detection of this invention, although there will be especially no limit if it comes out, the thing which is detection, identification, or the reagent that can carry out a quantum, and can fix the specific matter in a sample to said polymer by adsorption or association and which has functional groups, such as a carboxyl group and an amino group, is desirable. Protein is mentioned as a desirable reagent for detection. An antigen or an antibody is mentioned more to a detail. Although the reagent for detection of this invention is also fixable to the polymer layer directly described above, in fixing, both are also fixable using the matter which serves as a spacer if needed. Polyfunctional various matter can be used as a spacer. although the comparatively long-chain matter can also be used -- a carbon number -- 2-15 -- about two to ten short thing is preferably desirable. As a compound used as a spacer, dicarboxylic acid, such as halo carboxylic acids, such as alpha-bromoacetic acid and alpha-chloroacetic acid, a glutaric acid, an adipic acid, and a suberic acid, etc. is mentioned, for example.
[0028] A suberic acid is used for the metal thin film which has the polyethyleneimine film shown in drawing 3 at drawing 1 as a spacer, and how to fix the reagent for detection (shown as ligand in drawing 3 .) by the activity esterifying method is shown. How to fix the reagent for detection (shown as ligand in drawing 4 .) to CM-chitosan sensor probe shown in drawing 4 at drawing 2 is shown. EDC in drawing 4 shows a 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide, and NHS shows N-hydroxy Succin imide.
[0029] Gold, silver, copper, aluminum, chromium, etc. are mentioned that what is necessary is just the metal which has a free electron as a metal used for the metal thin film of this invention. Although a desirable metal is gold, it is a metal with desirable silver, copper, ARUMINIU, etc. A metal thin film extends a metal, may be manufactured and may be formed in a substrate by approaches, such as vacuum evaporationo. Although there will be especially no limit if the thickness of a metal thin film is measurable thickness, 50-500nm 50-1,000nm is about 100-500nm more preferably.
[0030] When using the metal thin film of this invention for surface plasmon resonance (Surface Plasmon Resonance:SPR) equipment, it is used for them to the metal thin film of this invention by substrates, such as prism, being stuck. The method of preparation of the metal thin film of this invention and the outline of surface plasmon resonance equipment are shown in drawing 5 . As shown in drawing 5 , considering the metal thin film of this invention as the method of preparation, there are a batch method and an all flow inject method (the AFI method). After a batch method processes a metal thin film by the required matter (shown as A liquid, B liquid, and C fluid in drawing 5 .) for making an organic linker layer, a polymer layer, the reagent for detection, etc. form and prepares the processed metal thin film (sensor probe), it is the approach of setting this in SPR equipment, and adding and measuring a sample.
[0031] Moreover, the AFI method processes the metal thin film set to the metal thin film set in SPR equipment by pouring a processing solution to a metal thin film one by one in the desired condition. As shown more in drawing 5 , in a detail, always with pumps, such as a pump for HPLC, the buffer solution etc. (For example, a running bar fur (pH7.4 10mMHBS(HEPES Buffer Saline)/Tueen)) The rate of flow can perform required processing by making a metal thin film (sensor probe) supply by 0.1-1.0, and passing by 0.2 - 0.4 ml/min preferably, and pouring in from an injector the solution which is the need and which contains each reaction reagent by the way. And it is also possible to continue measurement in the meantime, and the system of reaction can be measured, without irradiating measurement lines, such as direct light and microwave, for the reaction which has occurred in the opposite side of the measuring plane of a metal thin film at the system of reaction.
[0032] Since the AFI method can process the front face of a metal thin film, continuing measurement, it can grasp a surface condition on real time. Moreover, the mutual trends (chemical reaction etc.) of the reagent for detection fixed to the front face of a metal thin film and the matter in a sample can be measured serially, and can be observed. This invention includes any approach of a batch method and the AFI method.
[0033] In order to give the engine performance and function which are made into the purpose to an ingredient, it

becomes important to design an ingredient front face with a molecular level, and to give a functional characteristic. Therefore, an understanding of the reaction process which includes the interaction between the sensor material-list side and a biological substance in sensing of the antigen antibody reaction in today's clinical chemistry field and the biological substance using cel receptor recognition has been an important technical problem.

[0034] It aimed at building the sensor probe which can be used for immobilization of the broad matter, and detection by building a sensor front face using the macromolecule which has various functional groups in this invention. Using a golden thin film as a sensor base, surface plasmon resonance (Surface Plasmon Resonance:SPR) equipment was used as a device, and, specifically, the sensor front face was designed.

[0035] In fixing protein, in order to more specifically develop the high sensitivity immune sensor using the antigen antibody reaction, its attention was paid to the polyethyleneimine (polyethyleneimine (PEI)) which is the giant molecule which has a different functional group as film which embellishes a golden front face, and chitosan (chitosan). The chitosan film activated the carboxyl group for the primary amine to which the PEI film exists in the film, gave the amino group and binding site in protein, the chemical bond of the antigen was made to carry out there, and the sensor film was produced. In this invention, the all flow mold fixing method which introduces into a flow system all the reagents other than the batch method currently performed from the former, and fixes them as this production approach was also developed. The SPR angle-type shift at the time of an antibody combining with the produced sensor film specifically performed the quantum.

[0036] Moreover, in development of the new sugar sensor using sugar joint protein (Concanavalin A:ConA), the glucose was first made into the measuring object as sugar. As a property of ConA, since it has four binding sites with a glucose in the pH7 neighborhood, adsorbent [over a glucose] is high. However, ConA and a glucose were mixed beforehand, when what some binding sites combined with the glucose was made to act, alternative adsorbent [that] fell and this change considered that the concentration of ConA corresponded to fixed, then the glucose concentration to mix. This property was used and glucose concentration was conversely measured by embellishing Au front face with a glucose unit from the concentration of ConA which adsorbs there.

[0037] The approach of this invention is by the approach advantageous to measuring a reaction with the antigen or antibody especially fixed to the polymer layer through direct or a spacer, the antibody in a sample, or an antigen. For example, in order to fix antibody protein so much and to detect to high sensitivity, the polyethyleneimine (PEI) which is the giant molecule which has a different functional group as film material which embellishes a surface of metal, and especially carboxymethyl-ized chitosan (CM-chitosan) are desirable. The chemical bond of the ligand which CM-chitosan activates a carboxyl group and reacts specifically the primary amine to which PEI exists in the film with an antibody was carried out, and the sensor-probe front face was built.

[0038] the all flow injection (AFI) which in addition to the batch method currently performed from the former introduces all reagents into a flow system and fixes them in case these front faces are produced in this invention -- law was also examined. Specifically, the SPR response in a protein A-human immunoglobulin G (hIgG) and anti-human immunoglobulin G(anti-hIgG)-human immunoglobulin G (hIgG) system was considered using the produced sensor-probe.

[0039] The SPR sensor (product made from DKK) used for this experiment can perform the flow through system shown in drawing 5 . In experimenting by the AFI method, the running bar fur (pH7.4 10mMHBS(HEPES Buffer Saline)/Tueen) is always passed by the rate of flow 0.2 - 0.4 ml/min with the pump for HPLC, and by pouring in each sample solution from an injector, the sensor probe in SPR equipment is made to contact, and it is outputting the data by computer.

[0040] Next, although this invention is explained more concretely, this invention is not limited to these examples. The cable address used in more concrete explanation of this invention is shown collectively below.

[0041] Compound BSA; bovine serum albumin (Bovine serum albumin).
BS3; suberic-acid screw (sulfo SAKUSHIN imidoyl) (Bis (sulfosuccinimidyl) suberate.)
CAP; cellulose acetate phthalate (Cellulose acetate phtalate).
CM-chitosan; carboxymethyl chitosan (Carboxymeyhylatedchitosan).
EDC;1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide).
HBS;HEPES buffer solution (HEPES Buffer Saline).
hIgG; human immunoglobulin G (human immunoglobulin G).
NHS;N-hydroxy Succin imide (N-Hydroxysuccinimide).
PEI; polyethyleneimine (Polyethylene imine).

[0042] Solvent AcOH ; Acetic acid (Acetic Acid).
DMF; N.N-dimethylformamide (N and N-Dimethylformamide).
EtOH; ethanol (Ethanol).
i-PrOH; isopropanol (Isopropanol).
[0043] First, 5mM after washing by attaching a golden thin film to 1MKOH water solution warmed at about 70 degrees C several times It considered as the condition of (1) of drawing 1 by dipping in the DMF solution of L-cysteine methyl ester for 24 hours. This was processed in the glutaraldehyde water solution 5% for 2 hours, and was made into the condition of (2) of drawing 1 , and the showing [subsequently to a PEI water solution, dip 2% for 2 hours, and / by (3) of drawing 1] PEI film sensor probe was obtained.
[0044] Thus, the surface structure of the organic linker part of the prepared sensor probe has a thing in the condition of a part of GUTARU aldehydes having combined with the amino group of a dyad cysteine, and having consumed two aldehyde groups etc., and it is thought that the condition of differing from the condition of self-adsorbent monolayer (Self-Assembled Monolayer) that the molecule has aligned regularly in the surface of metal is formed. The condition of a detailed surface of metal could be grasped by using surface analysis, such as XPS, and measuring the thickness.
[0045] Next, the produced probe is set in SPR equipment and it is pH7.4. HBS It is 0.1M about the unreacted glutaraldehyde which exists in the film after the base line by T/buffer is stabilized. It blocked by **********ing a glycine solution. Then, the primary amine in the PEI film is activated by BS3 which is a spacer, and it is 0.5mg/ml. The result when fixing BSA is shown in drawing 6 , and, subsequently it is 0.1mg/ml. The result when adding hIgG is shown in drawing 7 .
[0046] Drawing 6 and drawing 7 are as a result of [of SPR] measurement, time amount (minute) is shown on an axis of abscissa, and a response (arc second (1 time is a 3600 arc second)) is shown on an axis of ordinate. (1) in drawing 6 is pH7.4. HBS The time of (4) adding a glycine pH 3 for the time of (3) adding 0.5mg [/ml] BSA for the time of (2) adding BS3 for the time of adding T/buffer is shown, respectively. Response deltatheta in the time of adding BSA was a 604 arc second. (1) in drawing 7 is pH7.4. HBS The time of (4) adding a glycine pH 3 for the time of (3) adding 0.1mg [/ml] hIgG for the time of (2) adding BS3 for the time of adding T/buffer is shown, respectively. Response deltatheta in the time of adding hIgG was a 150 arc second.
[0047] Next, processing of the metal thin film by the all flow inject method (the AFI method) was considered. After washing a golden thin film, it set in direct SPR equipment, and from there, all the reagents were introduced into the flow system and fixed. Namely, water of a 10mML-cysteine: Ethanol 1:4 solution, 10% glutaraldehyde water solution, and 1%PEI water solution were **********ed in order, the PEI film probe was produced, and BSA was fixed. A result is shown in drawing 8 and drawing 9 . (1) in drawing 8 is pH7.4. HBS The time of (4) adding PEI for the time of (3) adding glutaraldehyde for the time of (2) adding a 10mML(s)-cysteine for the time of adding T/buffer 10% 1% is shown, respectively.
[0048] (a) in drawing 9 is pH7.4. HBS The time of (e) adding the ethanolamine of 0.1M for the time of (d) adding a glycine pH 3 for the time of (c) adding 500microg [/ml] BSA for the time of (b) adding BS3 of 1mM for the time of adding T/buffer by pH8.5 is shown, respectively. It was the 1130 arc second which response deltatheta in the time of adding the glycine of (d) originates in an electrostatic interaction, and is a 334 arc second and originates in the interaction by covalent bond.
[0049] Next, control of the nonspecific adsorption to a PEI film sensor probe front face was considered. In case a sensor is produced, the nonspecific adsorption to the sensor film front face of the protein of the variety which exists in body fluid is pointed out as main causes of reducing detection sensitivity and its selectivity. Also in the above mentioned experiment, when contacting BSA on a PEI film front face, about 1/four to 1/3 of all the amounts of adsorption nonspecific adsorption (part from which it was desorbed when contacting a glycine / hydrochloric acid pH 3.0) was seen. It is thought that this is the causes with a main electrostatic interaction with BSA by the plus charge which it has in the film because the amine which remain protonates.
[0050] Then, it is pH7.4 in case protein is fixed in a PEI film probe. The result when changing pH of reaction time instead of HBST/buffer using pH5.3 acetic-acid buffer is shown in drawing 10 . (1) in drawing 10 shows the time of (4) adding the glycine (pH3.0) of 0.1M for the time of (3) adding a 0.5mg [/ml] BSA solution for the time of (2) carrying out 1mM addition of BS3 of a spacer for the time of adding pH5.3 acetic-acid buffer, respectively. Response deltatheta in the time of adding a glycine was 860 and a 620 arc second, respectively.
[0051] Moreover, by pH7.4 buffer, the amino group in the film is made to react with a 100mM griot kill acid (Glyoxylic Acid), without using BS3 as a spacer, it carboxylates, the charge balance of the whole film is maintained, and it is 0.4M about this. EDC/0.1M NHS After being activated with 1:1 mixed solutions, the result at

the time of fixing BSA is shown in drawing 11 . (1) in drawing 11 shows the time of (4) adding the glycine (pH3.0) of 0.1M for the time of (3) adding a 0.5mg [/ml] BSA solution for the time of (2) adding 0.4MEDC(s)/0.1MNHS for the time of adding a pH7.4HBS buffer, respectively. Response deltatheta at the time of adding BSA was a 1211 arc second, and response deltatheta at the time of adding a glycine was a 673 arc second.

[0052] Consequently, nonspecific adsorption of BSA was not able to be decreased by changing the charge balance of pH of reaction time, or a sensor probe front face. protein, such as BSA, -- as a whole -- one of the charges of plus or minus -- **** -- although it is in many cases -- the part of both the plus charges and minus charges to each intramolecular -- **** -- since it is, even if it maintains the balance of charge on the front face of a probe as a whole, it is difficult to change at least each part in a protein molecule into change of pH, or the condition of being hard to adsorb all on a film front face. Although optimal pH which prevents nonspecific adsorption, and a charge condition will be decided by the proteinic class, in case it measures as a PEI film immune sensor probe while the protein of varieties, such as inside of a blood serum, exists, the measurement which changes pH and charge balance does not have semantics at all.

[0053] Then, while making inactive the unreacted amino groups in the film with charge by constructing a bridge by glutaraldehyde and controlling charge, the increment in the active site by the aldehyde group which remained was considered. The antibody detected to the activation part of this probe and the ligand which reacts specifically were fixed, and this was made into the PEI film sensor probe. And it is shown in drawing 12 . [this reaction] [a ** type]

[0054] From the above experiment, it thinks as follows as a basic property of a PEI film sensor probe. the batch method with which production of a PEI film sensor-probe is performed from the former since ligand with the amino group was fixable -- in addition, the all flow injection (AFI) which introduces all reagents into a flow system and produces them -- it has checked that it could carry out also by law. Since the contact time of the sample and front face by the flow system was about 10 minutes, as for the reaction shown in drawing 1 , it turned out that all advance within this time amount. The AFI method is considered to be an approach useful as the production approach of a simple and quick probe as compared with other sensor probes which take dozens hours for production established until now. A comparison of the amount of immobilization of BSA of the PEI film probe produced with the batch and the probe by the flow detects the direction of a flow system as a 2.8 times bigger response (drawing 6 and drawing 8 , drawing 9). However, the direction of a flow system is small and the response of BS3 which is an activator for BSA immobilization has become the amount of immobilization and reverse of BSA. It is thought that it originates in a certain difference being shown in the surface structure of two created probes.

[0055] Moreover, in order to make small the nonspecific amount of adsorption of the protein to the PEI film front face which poses a problem, it is necessary to contact BS3 and EDC/NHS of the highest possible concentration, and to fully block activating the functional group in the film, and the surplus amino group. Introducing a reagent with the ethylene glycol unit including many ether linkage known as matter with little adsorption of protein in the case of the design on the front face of the film is also considered as one approach.

[0056] Next, as application of a sensor probe, anti-hIgG and proteinA were combined with the binding site in the PEI film, the response when changing concentration and contacting the immunoglobulin antibody of human being who has high singularity and strong bonding strength in these, and hIgG was measured, respectively, and the quantum of hIgG was performed. Moreover, the amount of non-specific adsorption of the protein on the front face of a probe was examined by contacting mouse-IgG with weak coupling nature with proteinA, and the chicken IgG (chicken-IgG) which does not show affinity at all. Eight kinds of probes used for measurement are packed into drawing 13 .

[0057] The real-time plot of the hIgG detection when measuring using the probe of No.8 of drawing 13 first was shown in drawing 14 . (1) in drawing 14 the time of adding a HBS/NHS buffer (2) the time of (3) adding anti- [200microg //ml] hIgG for the time of adding EDC/NHS (4) shows the time of (7) adding 1.0 ug(s)/ml hIgG for the time of (6) adding 0.1 ug(s)/ml hIgG for the time of (5) adding a glycine for the time of adding ethanolamine, respectively.

[0058] When EDC/NHS was **********ed in the condition that the buffer is carrying out steady flow (at the time of (2) of drawing 14), the carboxyl group in the film was activated and anti-hIgG was contacted, the amount of immobilization like 2100arcsecond was seen in the SPR angle-type shift. While desorbing anti-hIgG to which it is sticking by charge with pH8.5 ethanolamine and pH3.0 glycine solution at the time of (4) of drawing 14 , and (5), the activation part in the remaining film was blocked. Production of the sensor probe for hIgG measurement was completed even here, concentration was changed, hIgG was contacted on the film, and the response

6btained was detected. First, 0.1microg/ml It is 288arc, when the hydrochloric acid was poured, the membranous condition was returned and ml was contacted in 1microg /of one 10 times the concentration of this, since it was hardly detected as a result of **********ing hIgG. The response of second was detected. 60 and 160 -- again -- 60microg/ml As a result of **********ing hIgG in order, the response corresponding to each concentration was able to be obtained (refer to drawing 15). [then,]

[0059] The mistake was made in having created the calibration curve based on the data obtained for every probe, and being based on the activation approach (No.2-No.6 of drawing 13 , No.4-No.8), and it examined making a mistake in being based on the production approach (No.5-No.6 of drawing 13 , No.7-No.8) (refer to drawing 16 and drawing 17). The left-hand side in drawing 16 is based on the batch method activated by BS3, right-hand side is based on the batch method activated by EDC/NHS, the black trigonum mark and the white square mark show a protein A-hIgG system, and the black dot mark and the BATSU mark show an anti-hIgG-hIgG system. The left-hand side in drawing 17 is based on the batch method activated by EDC/NHS, the AFI method activated by EDC/NHS depends right-hand side, the white square mark and a white round mark show a protein A-hIgG system, and the BATSU mark and the white rhombus mark show an anti-hIgG-hIgG system. moreover -- the amount of adsorption of a non-specific antibody -- No. -- it inquired by comparing 1, 5, and 6 chip (refer to drawing 18). The rod of the gray in drawing 18 shows hIgG, the rod of a slash shows Mouse IgG, and the rod of striping shows Chicken IgG. Any concentration is 90microg/ml. The amino group in the PEI film was carried out to the result of having been activated using BS3, with the glyoxylic acid at the carboxyl group, tea-JIBARANSU on the whole front face of the film was taken, and comparison examination of this was carried out by drawing 16 about the case where it is activated by EDC/NHS.

[0060] Consequently, in the protein A-hIgG system, it turned out that detection sensitivity also increases to the top where the linearity of the calibration curve from which the direction of the front face which introduced the carboxyl group is obtained is high. Even if this sees the amount of adsorption of the non-specific antibody of drawing 18 , as compared with the BS3 activity of No.1, mouse (Mouse) and chicken (Chicken)-IgG is seen relatively [amount / of hIgG], the amount of adsorption is pressed down, and its direction of the EDC/NHS activity of probe No.5 corresponds also with the result to which detection precision became high. In the above mentioned experiment of immobilization of BSA, although the nonspecific amount of adsorption of BSA increased by introducing a carboxylic acid into PEI, in this antigen antibody reaction, the difference in the complicated structure of protein, such as the isoelectric point, showed that the non-specific adsorption to the film of mouse (Mouse) and chicken (Chicken)-IgG could be prevented by introducing charge of minus into the film.

[0061] By drawing 17 , it divided with the AFI method and the batch method about the system which introduced this carboxylic acid, and the calibration curve was produced. Consequently, detection sensitivity equivalent to a batch method and linearity were able to be acquired also about the probe produced by the flow system. Moreover, as shown in drawing 18 , as for the PEI film produced by the AFI method, nonspecific adsorption decreased as compared with the batch method. It is thought that this is because a certain difference is in the membrane structures on the front face of a chip (stacking tendency of the amino group etc.) (refer to drawing 19). For example, as shown in 19 Fig., an adsorption homopolymer mold, an anchoring mold polymer (cerminally-anchored) mold, etc. can be considered. Immobilization of the protein to the metal by the AFI method, especially a golden base front face does not have an example of a report until now, and it is thought that it is an approach useful as a method of producing simplicity and a quick sensor probe with sufficient repeatability. It turns out that control of the charge on the front face of the film is one important factor at the design on the front face of the film with less proteinic nonspecific adsorption than these results, and a detection property and detectivity improve further by examining film material and the production approach further.

[0062] Moreover, if protein generally contacts a macromolecule front face, it will increase a contact gradually, the conformation is sometimes changed, and the property of a natural condition is spoiled. Furthermore, activity will be dropped, even if the active site is a contact with a front face, or it is arranged so that a failure may be carried out in three dimensions. Controlling the stacking tendency in the case of ligand immobilization also leads to the increment in detection precision.

[0063] Next, it examined using the polymer containing aminosugar for the film. It is Pori aminosugar, as for chitosan (chitosan), the acetyl group of a chitin was hydrolyzed partially, and a chitin (chitin) is beta of D-glucose amine. -(1 4)- It is a polymer. Since chitin and chitosan are the organization base materials of crustacean and Insecta, compatibility with a living body is good and is decomposed by the enzyme in the living body. It is used for the absorbable surgical suture, the artificial skin, etc. as a medical ingredient using this. Moreover, since there is little adsorption of components in body fluid, such as having immunity activity and

protein, it is used also as support of a drug delivery system. Furthermore, since the derivative of chitosan has alternative adsorbent [of an optical isomer] with the die length of an acyl group, it is used for separation of racemic mixture by HPLC.

[0064] By this invention, production of a chitosan film immune sensor probe was tried using the high biocompatibility of chitosan, and a property without the nonspecific adsorption of the protein which became a problem with the PEI film sensor probe. First, 5mM after washing by attaching a golden thin film to 1M warmed at about 70 degrees C, and a KOH water solution several times like the time of PEI After dipping in the DMF solution of L-cysteine methyl ester for 24 hours, this was soaked in the glutaraldehyde water solution 5% for 2 hours. Chitosan was fixed by contacting a chitosan water solution to this 1% for 24 hours. Next, it carboxylated by dipping the OH radical in chitosan in BrCH2COOH / 1M NaOH solution of 2M for 12 hours, and the carboxymethyl-ized chitosan (CM-chitosan) sensor probe was produced (refer to drawing 2).

[0065] The produced probe is set in SPR equipment like measurement by the PEI film probe, and it is pH7.4. HBS It is 0.4M about -COOH radical in the film after the base line by T/buffer is stabilized. EDC/0.1M NHS 0.5mg/ml after **********ing 1:1 mixed solutions and being activated The result when fixing BSA is shown in drawing 20 . (1) in drawing 20 shows the time of (4) adding ethanolamine for the time of (3) adding a 0.5mg [/ml] BSA solution for the time of (2) adding 0.4MEDC(s)/0.1MNHS for the time of adding a pH7.4 HBS/T buffer, respectively. Response deltatheta at the time of adding BSA was a 150 arc second.

[0066] After making - OH radical in the film into -COOH radical by dipping BrCH2COOH in the chitosan film and activating this, when BSA was fixed, as shown in drawing 20 , the response of a 150 arc second was obtained. It is thought in that case that it depends for the amount of immobilization of BSA on the amount of the COOH radical which exists in a CM-chitosan film probe. Then, when carboxylating chitosan, the increment in the amount of immobilization of ligand was tried by compounding CM-chitosan with the high rate of carboxylation beforehand, and fixing this on a front face from what is performed on a probe front face with a batch method.

[0067] Chitosan 1.0g and lauryl sulfonic-acid sodium 0.1g were slowly added into 70ml of 45%NaOH water solutions, and it stirred for about 1 hour until it dissolved keeping at 4 degrees C. After cooling this radiationally at -20 degrees C for about 12 hours, i-PrOH125ml and ClCH2COOH28.4g were added and it was made to react for 72 hours. Then, 2MHCl is added and 9.8727g of white crystals was obtained. Next, after dissolving 0.5g of this crystal in 20ml of NaOH water solutions 10%, 4MHCl was added until it was set to pH1. The crystal was deposited by adding a lot of acetones to this, and 0.7g CM-chitosan was obtained by suction filtration.

[0068] Although it fixed in the sensor probe by the same approach as the scheme which described above CM-chitosan compounded by the aforementioned approach the front and the carboxyl group was activated, by this system, BSA was unfixable. It is thought that there is a step for which a reaction does not go into a scheme. Since it turned out that a reaction progresses, immobilization of glutaraldehyde was expected that the reaction which fixes CM-chitosan in mono-layer - is a neck. Since the amino group of sugar is also carboxylated in case CM-chitosan is compounded, it is in a polymer. - It is thought that there is no degree of freedom since the amount of two NH(s) having decreased and two -NH(s) are directly attached to the principal chain of a polymer, and it has become a cause that reactivity is low. Then, it changed into another reaction trajectory and the chitosan film sensor-probe was produced.

[0069] It is 2 hours and 0.4M to the HS-C10H 20 COOH/EtOH solution of 10-3M after washing a golden thin film. EDC/0.1M After dipping in 1:1 mixed solutions of NHS for 40 minutes, the chitosan film sensor probe II was produced by attaching to previous 2%CM-chitosan water solution for 2 hours. This probe is used and it is 0.4M about -COOH radical in the film. EDC/0.1M NHS It is activated with 1:1 mixed solutions and is 200microg/ml. After fixing hIgG, the result of having measured adsorption on the film of 500microg [/ml] BSA is shown in drawing 21 . (1) in drawing 21 shows the time of (5) adding 500microg [/ml] BSA for the time of (4) adding ethanolamine for the time of (3) adding a 0.2mg [/ml] IgG solution for the time of (2) adding EDC/NHS for the time of adding a pH7.4 HBS/T buffer, respectively. Response deltatheta at the time of adding BSA was a 720 arc second.

[0070] By changing the system of reaction, it was fixable based on CM-chitosan. Most nonspecific adsorption from the property of chitosan to the film of the protein (BSA) seen by the PEI film in the CM-chitosan film probe was not seen. In order to confirm it, chitosan and CM-chitosan are fixed in a probe, and the situation when contacting BSA without activating them is shown in drawing 22 and drawing 23 . (3) shows [the time of (2) adding a glycine/HCl (pH3) for the time of (1) in drawing 22 adding a pH7.4 HBS/T buffer] the time under addition for 500microg [/ml] BSA, respectively. Response deltatheta at the time of adding BSA was 50 arc second. (1) in drawing 23 shows the time of (2) adding 500microg [/ml] BSA for the time of adding a pH7.4

controls nonspecific adsorption of protein, and the same inclination was seen also with CM-chitosan and a CMD film probe.

[0079] Although it has discussed adsorbent [proteinic] from the charge and the point of relative-degree-of-intimacy aquosity, I want to consider this specific property from a viewpoint of the water near a front face, and a membranous interaction until now. From the importance as a solvent indispensable to a life, the consistency became high most near 4 degree C, and water has for many years very often been studied for the specific property to have the remarkable big specific heat compared with other liquids. Although the property of this water originates in the "structure" formed when molecules carry out hydrogen bond mutually, many unsolved problems are left behind about that structure. Moreover, it is known conversely that water soluble polymers including protein or a macromolecule receive effect in the structure and property, and a function greatly by the interaction with water or that the water of hydration of a macromolecule and the water in polymer gel differ from bulk water in properties, such as maneuverability and freezing-fusion behavior. Generally, the water on the front face of a macromolecule can be classified into free water and the water bound to the polymer, and the conditions of the hydration sphere formed in a polymer front face of the strength of restraint differ. The condition of this surface hydration sphere is said to be one of the factors which determines adsorbent [to a proteinic front face].

[0080] As a point common to the chemical structure of a polyethylene glycol, CM-chitosan, and CMD, each has ether linkage in the interior of a molecule, and the hydration sphere formed in a film front face of this part is expected whether to control nonspecific adsorption of protein.

[0081] the batch method of the former [this invention] as a method of producing a PEI film sensor probe -- adding -- all flow injection (AFI) -- high response sensibility equivalent to a batch method was obtained, and the amount of adsorption of a non-specific antibody of the PEI film which offered law and was produced by the AFI method was also small. Moreover, it turns out that control of the charge on the front face of the film is one important factor at the design on the front face of the film with little proteinic nonspecific adsorption, and it is thought by examining film material and the production approach further that a detection property and detectivity improve further. Furthermore, as compared with the batch method, as for the PEI film produced by the AFI method, detection precision was also found by becoming high the top with little nonspecific adsorption. This is considered to be because for a certain difference to be in the membrane structures on the front face of a probe (stacking tendency of the amino group etc.). Immobilization of the protein to the surface of metal by the AFI method does not have an example of a report until now, and is considered to be an approach useful as a method of producing simplicity and a quick sensor probe with sufficient repeatability.

[0082] In the examination of a sensor probe based on the CM-chitosan film of this invention, production by the AFI method and the batch method was performed, protein A was fixed, and hIgG was detected. Consequently, although response sensibility fell about to 1/4 as compared with the case of the PEI film, as for the CM-chitosan film, nonspecific adsorption was not seen at all. It turns out that control of the charge on the front face of the film is one important factor at the design on the front face of the film with less proteinic nonspecific adsorption than these results, and it is thought by examining film material and the production approach further that a detection property and detectivity improve further.

[0083] In order to clarify effectiveness of the sensor probe of this invention more, the comparative study with a well-known dextran film sensor chip was performed. Since the dextran film was already carboxymethyl-ized, it was activated with 1:1 mixed liquor of 0.4MEDC(s)/0.1MNHS like the PEI film of this invention, and fixed protein A to both. Concentration 0.1, 1.0 and 10, and 45 or 90,200microg [/ml] hIgG were passed in each sensor at this. Aging of the PEI film of this invention is shown in drawing 27 . Moreover, both calibration curve acquired in these experiments is shown in drawing 28 . The left-hand side of drawing 28 is the thing of the PEI film of this invention, and right-hand side is the thing of the dextran film.

[0084] Consequently, by the PEI film of this invention, it turns out that hIgG concentration shows linearity mostly in the 10-200microg [/ml] range by 0.1-90microg [ml] /and the dextran film, and the PEI film of this invention is about 20 times larger in that response sensibility, therefore limit of detection is also low so that drawing 28 may also show. Thus, when the polymer which has the comparatively easy chemical structure is used for the polymer film, especially PEI film of this invention shows that simple and cheap it is moreover the sensor probe of high sensitivity.

[Example] Next, although an example explains this invention still more concretely, this invention is not limited to these examples.

[0085] 5mM after washing by attaching the 1 carat thin film of examples to 1MKOH water solution warmed at

HBS/T buffer, respectively. Response deltatheta at the time of adding BSA was a 100 arc second.
[0071] The response when becoming in 500 ug/ml, although a lot of [CM-chitosan whose chitosan is the derivative although most non-specific adsorption was not seen as the property] carboxyl groups in the film exist, and contacting high-concentration BSA became a 100 arc second and a very small value. BURROKU of the charge on the front face of a probe which this performed in the PEI film probe production course has checked that it was unnecessary by the CM-chitosan film.
[0072] Next, the chitosan film sensor probe by the AFI method was prepared like PEI. The golden thin film was set in SPR equipment like the time of a PEI film sensor probe, and the all flow system fixing method which introduces all reagents into a flow system and fixes them from there was tried. A result is shown in drawing 24 . (1) in drawing 24 shows the time of (5) adding 0.5mg [/ml] BSA for the time of (4) adding 1%CM-chitosan for the time of (3) adding 0.2M/0.05M of EDC/NHS for the time of (2) adding the ethanol solution of a mono-layer for the time of adding a pH7.4 HBS/T buffer, respectively. Response deltatheta at the time of adding BSA was observed.
[0073] although the system performed by the flow was also able to fix BSA as a result of trying production of a CM-chitosan film sensor-probe by the flow system -- the response ** -- it was small. This is considered that there is a step whose reaction is not enough in time from the contact time on each reagent in a flow system and the front face of the film being about 10 minutes. It is in CM-chitosan which is the fixed part where it was expected that the reaction which fixes CM-chitosan in mono-layer - was a neck since the PEI film in a flow is producible, and this probably became a problem. - It is thought that there is no degree of freedom since two NH (s) are directly attached to the principal chain of a polymer, and it has become a cause that reactivity is low. It is thought by embellishing a polymer so that it may have functional groups, such as an amino group with a big degree of freedom, in not a principal chain but the side chain of a polymer that it can consider as the polymer which has reactivity more.
[0074] From the old experimental result, production of the sensor probe fixed based on compound CM-chitosan could carry out with the batch method, and the detection with the amino group of ligand has checked as a SPR signal. Most nonspecific adsorption to the film of the protein (BSA) seen by the PEI film probe was not seen at that time. Although it is also applicable to the AFI method of this probe, since two -NH(s) are directly attached to the principal chain of a polymer, there is no degree of freedom, it becomes a cause that reactivity is low, and sufficient reactivity may not be seen compared with the batch way. CM-chitosan composition -- already -- it is thought also by introducing the high SU **-sir of a hydrophilic property into one step, in addition the amino group, and raising the degree of freedom of a binding site with a mono-layer that this problem is solvable.
[0075] Moreover, it is one of the troubles that the water solubility of CM-chitosan is also low, when producing a probe. It turns out that water solubility increases as chitosan generally has hundreds of thousands of molecular weight and this becomes small. Chitosan with small molecular weight is used and it is expected that a base becomes is easy to be fixed by compounding CM-chitosan. If acidity is too high as a reaction condition at that time, since it will be lost because the nucleophilicity of the amino group protonates and a reaction will not progress, it is thought more appropriate than neutrality to react by the basicity side a little.
[0076] Furthermore, this invention offers the high sensitivity immune sensor using the antigen antibody reaction. As application of a CM-chitosan sensor probe, in order to compare with the PEI film, protein A was combined with the binding site, the response when changing concentration and contacting the immunoglobulin antibody of human being who has high singularity and strong bonding strength in these, and hIgG was measured, respectively, and the quantum of hIgG was performed. No. -- the calibration curve doubled with the result of the PEI film probe of 5 and 6 is shown in drawing 25 . The round mark in drawing 25 is based on the AFI method which used the PEI film probe, the square mark is based on the batch method which used the PEI film probe, and the trigonum mark is based on the batch method which used CM-chitosan film probe.
[0077] Moreover, the amount of non-specific adsorption of the protein on the front face of a probe is examined by contacting similarly mouse-IgG with weak coupling nature with protein A, and chicken (chicken)-IgG which does not show affinity at all, and the data in comparison with the CMD film probe which are PEI and a commercial item are shown in drawing 26 . The rod of the gray in drawing 26 shows hIgG, the rod of a slash shows Mouse IgG, and the rod of striping shows Chicken IgG. Any concentration is 90microg/ml.
[0078] Although response sensibility fell about to 1/4 as compared with the PEI film, even if adsorption of a nonspecific antibody was not regarded as having considered this film before at all but having been compared with the CMD film probe which is a commercial item, a high detection precision was able to be acquired. Until now, it is reported that the probe by which clothing was carried out in the front face with the oligo ethylene mono-layer

500microg [/ml] BSA is shown in drawing 21 .

[0095] The chitosan film sensor probe was prepared by the AFI method like example 11 example 2. The golden thin film was set in SPR equipment like the time of a PEI film sensor probe, and the fixing method was performed by the all flow system which introduces all reagents into a flow system and fixes them from there. A result is shown in drawing 24 . (1) in drawing 24 shows the time of (5) adding 0.5mg [/ml] BSA for the time of (4) adding 1%CM-chitosan for the time of (3) adding 0.2M/0.05M of EDC/NHS for the time of (2) adding the ethanol solution of a mono-layer for the time of adding a pH7.4 HBS/T buffer, respectively. Response deltatheta at the time of adding BSA was observed.

[0096] The example 12PEI film was activated with 1:1 mixed liquor of 0.4MEDC(s)/0.1MNHS, and protein A was fixed to this. Concentration 0.1, 1.0 and 10, and 45 or 90,200microg [/ml] hIgG were passed in each sensor at this. The result of aging of the PEI film by the AFI method is shown in drawing 27 . 1,000R.U in drawing 27 is 2 1 ng/mm. Moreover, the calibration curve acquired in this experiment is shown in the left-hand side of drawing 28 .

[0097] It carried out like the example 12 using the example of comparison 1 dextran film. The calibration curve acquired as a result is shown in the right-hand side of drawing 28 .

[0098]

[Effect of the Invention] This invention can offer simplicity and a quick and metal thin film useful as a sensor probe for SPR measurement with sufficient repeatability, the sensor probe of this invention is high sensitivity, and it can be highly precise, and can be simple, and it can be manufactured cheaply.

[Translation done.]

about 70 degrees C several times It considered as the condition of (1) of drawing 1 by dipping in the DMF solution of L-cysteine methyl ester for 24 hours. This was processed in the glutaraldehyde water solution 5% for 2 hours, and was made into the condition of (2) of drawing 1 , and the showing [subsequently to a PEI water solution, dip 2% for 2 hours, and / by (3) of drawing 1] PEI film sensor probe was obtained.

[0086] The probe produced in the example 2 example 1 is set in SPR equipment, and it is pH7.4. It is 0.1M about the unreacted glutaraldehyde which exists in the film after the base line by HBST/buffer is stabilized. It blocked by **********ing a glycine solution. Then, the primary amine in the PEI film is activated by BS3 which is a spacer, and it is 0.5mg/ml. The result when fixing BSA is shown in drawing 6 , and, subsequently it is 0.1mg/ml. The result when adding hIgG is shown in drawing 7 .

[0087] It is pH7.4 in case protein is fixed in a PEI film probe in example 3 example 2. HBS pH of reaction time was changed instead of T/buffer using pH5.3 acetic-acid buffer, and it carried out like the example 2. The result was shown in drawing 10 .

[0088] It sets in an example 4 and the example 2, and is pH7.4. At buffer, they are 100mM(s) as a spacer instead of BS3. It is made to react with GURIOKISHI acid RU (OHC-COOH), a carboxyl group is introduced, the charge balance of the whole film is maintained, and it is 0.4M about this. EDC/0.1M NHS BSA was fixed after being activated with 1:1 mixed solutions. This result was shown in drawing 11 .

[0089] When EDC/NHS was **********ed in the condition that the sink and the buffer are carrying out steady flow of the buffer solution to the sensor probe which introduced the carboxyl group with example 5 spacer, the carboxyl group in the film was activated and anti-hIgG was contacted, the amount of immobilization like 2100arcsecond was seen in the SPR angle-type shift. Subsequently, while desorbing anti-hIgG to which it is sticking by charge with pH8.5 ethanolamine and pH3.0 glycine solution, the activation part in the remaining film was blocked. Here, production of the sensor probe for hIgG measurement was completed. Concentration was changed, hIgG was contacted on the film and the response obtained was detected. It is 288arc when ml was contacted in 1microg /. The response of second was detected. 60 and 160 -- again -- 60microg/ml As a result of **********ing hIgG in order, the response corresponding to each concentration was able to be obtained. [then,] The result was shown in drawing 14 and drawing 15 .

[0090] an example 6 -- the mistake was made in having created the calibration curve based on the data obtained like the example 5 for every probe, and being based on the activation approach (No.2-No.6 of drawing 13 , No.4-No.8), and it carried out similarly about making a mistake in being based on the production approach (No.5-No.6 of drawing 13 , No.7-No.8). The result was shown in drawing 16 and drawing 17 as a calibration curve. moreover -- the amount of adsorption of a non-specific antibody -- No. of drawing 13 -- it inquired by comparing 1, 5, and 6 chip. The result was shown in drawing 18 .

[0091] 5mM after washing by attaching a golden thin film to 1M warmed at about 70 degrees C, and a KOH water solution several times like the time of PEI of example 7 example 1 After dipping in the DMF solution of L-cysteine methyl ester for 24 hours, this was soaked in the glutaraldehyde water solution 5% for 2 hours. Chitosan was fixed by contacting a chitosan water solution to this 1% for 24 hours. Next, it carboxylated by dipping the OH radical in chitosan in BrCH2COOH / 1M NaOH solution of 2M for 12 hours, and the carboxymethyl-ized chitosan (CM-chitosan) sensor probe was produced (refer to drawing 2).

[0092] The produced probe is set in SPR equipment like measurement by the example 8PEI film probe, and it is pH7.4. HBS It is 0.4M about -COOH radical in the film after the base line by T/buffer is stabilized. EDC/0.1M NHS 0.5mg/ml after **********ing 1:1 mixed solutions and being activated The result when fixing BSA is shown in drawing 20 .

[0093] Example 9 chitosan 1.0g and lauryl sulfonic-acid sodium 0.1g were slowly added into 70ml of 45%NaOH water solutions, and it stirred for about 1 hour until it dissolved keeping at 4 degrees C. After cooling this radiationally at -20 degrees C for about 12 hours, i-PrOH125ml and ClCH2COOH28.4g were added and it was made to react for 72 hours. Then, 2MHCl is added and 9.8727g of white crystals was obtained. Next, after dissolving 0.5g of this crystal in 20ml of NaOH water solutions 10%, 4MHCl was added until it was set to pH1. The crystal was deposited by adding a lot of acetones to this, and 0.7g CM-chitosan was obtained by suction filtration.

[0094] It is 2 hours and 0.4M to the HS-C10H 20 COOH/EtOH solution of 10-3M after washing the 10 carat thin film of examples. EDC/0.1M After dipping in 1:1 mixed solutions of NHS for 40 minutes, the chitosan film sensor probe II was manufactured by attaching to 2%CM-chitosan water solution manufactured in the example 9 for 2 hours. This probe is used and it is 0.4M about -COOH radical in the film. EDC/0.1M NHS It is activated with 1:1 mixed solutions and is 200microg/ml. After fixing hIgG, the result of having measured adsorption on the film of